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14. ABSTRACT

Increased receptor recycling leads to elevated receptors on the plasma membrane, thereby augments signaling cascades important in cancer progression and drives tumor aggressiveness. Inhibition of the altered recycling pathway in breast cancer cells would thus dampen oncogenic signaling and increase the efficacy of therapy. We propose to monitor EGFR trafficking using a newly developed AP-tag (or Avitag) system. We have generated functional constructs for the label of EGFR using AP-tag and established the labeling technique in the lab. Furthermore, we also generated several stable cell lines expressing the AP-EGFR.

15. SUBJECT TERMS
Breast cancer, receptor, recycling, AP-tag, and EGFR.

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Table of Contents

Introduction:

Several recycling regulatory proteins, *e.g.*, Rab11A, Rab25, RCP and Rab11FIP5, are overexpressed in breast cancer patients and promote the progression of breast cancer. Increased receptor recycling leads to elevated receptors on the plasma membrane (PM), thereby augments signaling cascades important in cancer progression and drives tumor aggressiveness. Some ErbB2 targeting drugs inhibit breast cancer partially through shunting the receptor away from recycling into lysosomal degradation. However, the inherent recycling tendency of ErbB2 limits the effectiveness of treating cancer cells with their inhibitors. Inhibition of the altered recycling pathway in breast cancer cells would thus dampen oncogenic signaling and increase the efficacy of therapy. However, Rabs and their binding-proteins are traditionally hard to target. Identification of "druggable" proteins regulating receptor recycling is needed to target this distinct hallmark of breast cancer. A recently developed method, AP-tag (or Avitag), offers specific and covalent attachment of biotin by *E. coli* biotin ligase (BirA) to a surface protein of interest fused with only a 15-amino acid peptide, providing simplicity and extraordinary versatility to study the trafficking of surface proteins in live cells. We propose to develop an AP-tag system to monitor EGFR trafficking. We will then screen proteins responsible for dysregulated receptor trafficking in breast cancer cells and determine the role of these proteins in breast cancer cell proliferation, survival and migration/invasion.

This is a high-risk proposal. However, the relevance to breast cancer is high and the potential reward is great. The success of the proposal will provide new therapeutic targets from breast cancer.

Body:

We have expressed and purified BirA required for AP tag detection. After generated several constructs with AP-tag at different locations of the EGFR N-terminus, we have successfully identified one of the best construct for labeling and have set up to use the AP-tag to follow the endocytosis and recycling of EGFR (Figure 1). One challenge for library screening is heterogeneous responses. Thus a stable cell line from a single colony would help to reduce the heterogeneity during siRNA library screening. Although it is time consuming to generate a good cell line, we have picked 1-2 good AP-EGFR expressing cell lines for our screening (Figure 2). Some data are summarized below.

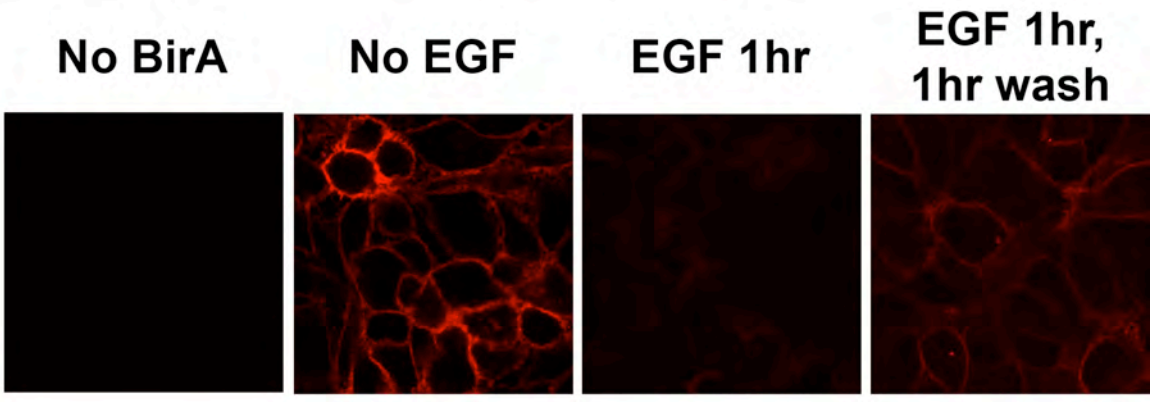

Figure 1. The AP-EGFR on the cell surface can be labeled by biotin and Alexa 594-conjugated streptavidin. MDA-MB-231 cells were infected with lentiviruses expressing AP-EGFR and selected with puromycin. The surface AP-EGFR was visualized by biotin/Alxa 594-strepvatavidin without cell permeabilization. Cells expressing AP-EGFR were visualized in the following conditions: unstimulated, stimulated with EGF for 30 min (internalization), or 1 hr after washing out EGF (pre-treated cells with EGF for 1 hr).

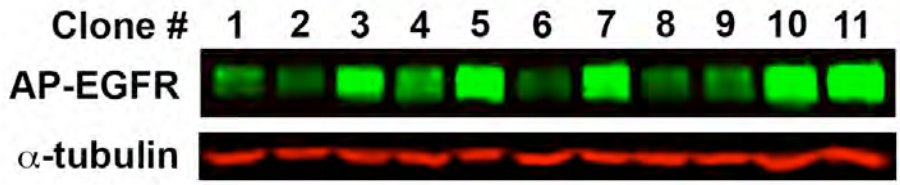

Figure 2. Establishment of MDA-MB-231 cells stably expressing different levels of AP-EGFR. MDA-MB-231 cells expressing AP-EGFR were serially diluted. Stable cell lines expressing different levels of AP-EGFR were established from individual clones. The AP-EGFR was detected with an AP tag antibody followed by the IRDye800 anti-mouse secondary antibody, and visualized using a LI-COR Odyssey imaging system.

The project was slower than expected mainly because we got the incorrect AP-EGFR construct from the investigator who originally reported the AP tag technique. Since this construct was used in several publications, we used it to generate stable cell lines directly. However, after spending 2-3 months on generating stable cell lines, we found out that this construct did not make a full-length EGFR protein. It turned out there was a point mutation in the construct that made the protein truncated. We spent a lot of time

on trouble shooting and re-making new constructs in our lab. Fortunately, the new construct we generated appears to behave as expected.

Key Research Accomplishments

- Corrected the mutation in the plasmid obtained from the original inventor.
- Tested several EGFR constructs with the AP tag at different locations.
- Set up the labeling protocol in the lab.
- Established stable cell lines expressing AP-EGFR.

Reportable Outcomes

The research progress was reported as an abstract and a poster at the 2011 DOD Era of Hope meeting. Several stable cell lines expressing AP-EGFR were created.

Conclusion

The AP-EGFR would allow us to follow the endocytosis and recycling of EGFR. We are working on optimalizing the conditions for siRNA library screening. The signal intensity might be a potential problem.

References

N/A

Appendices

None

Supporting data

None